Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/002017

International filing date: 20 January 2005 (20.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/531,168
Filing date: 20 January 2004 (20.01.2004)

Date of receipt at the International Bureau: 03 March 2005 (03.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
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February 16, 2005

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APPLICATION NUMBER: ***60/531,168***
FILING DATE: ***January 20, 2004***
RELATED PCT APPLICATION NUMBER: ***PCT/US05/02017***

Certified by

W. Dudas

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PTO/SB/16 (06-03)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 117250566 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Franklin W.	Okumu	San Diego, CA, USA

Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Treatment of Degenerative Cartilage Conditions in a Mammal with Glycosidase Inhibitors.

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS ***(check all that apply)***

[X] Specification *Number of Pages* 21
[ ] CD(s), Number
[ ] Drawing(s) *Number of Sheets*
[ ] Other (specify)
[ ] Application Date Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X] Applicant claims small entity status. See 37 CFR 1.27.
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FILING FEE Amount ($): $ 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.
[ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE

TYPED or PRINTED NAME Thomas E. Jurgensen, Esq.

TELEPHONE 858-450-0099

Date December 19, 2003

REGISTRATION NO. 34,195 *(if appropriate)*

Docket Number: 8024-014-PR

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PROVISIONAL APPLICATION COVER SHEET
Additional Pag

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Docket Number 8024-014-PR

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Number 1 of 1

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December 19, 2003

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12/19/03
Date of Deposit

EV117250566US

Mail Stop: PROVISIONAL PATENT APPLICATION
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Re: TREATMENT OF DEGENERATIVE CARTILAGE CONDITIONS IN A MAMMAL WITH GLYCOSIDASE INHIBITORS
Our Docket No.: 8024-014-PR

Dear Sir or Madam:

Enclosed please find the following documents related to the above-identified matter:

1. Provisional Application for Patent Cover Sheet (PTO/SB/16);
2. Fee Transmittal (PTO/SB/17);
3. Application Document (21 pages); and
4. Self-addressed, stamped postcard.

The self-addressed, stamped postcard has been included for your convenience. After confirming receipt of these documents please return the postcard to us at your earliest convenience. Should you have any questions, please do not hesitate contacting me.

Sincerely yours,

Thomas E. Jurgensen, Esq.
Reg. No. 34,195

Enclosures

F:\Clients\8024 Optimer Pharmaceuticals, Inc\014-PR\To File\Provisional to PTO.ltr.doc

PTO/SB/17 (05-03)
Approved for use through 04/30/2003. OMB 0651-0032
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FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete If Known	
Application Number	
Filing Date	December 19, 2003
First Named Inventor	Okumu, Franklin W.
Examiner Name	
Art Unit	
Attorney Docket No.	8024-014-PR

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☑ Deposit Account:

Deposit Account Number: 502235

Deposit Account Name: BKF Jurgensen

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

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2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below	Fee Paid
Total Claims		-20** =		X =
Independent Claims		- 3** =		X =
Multiple Dependent				=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

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SUBTOTAL (3) ($)

SUBMITTED BY				(Complete *(if applicable)*)	
Name (Print/Type)	Thomas, E Jurgensen, Esq.	*Registration No. (Attorney/Agent)*	34,195	*Telephone*	858-450-0099
Signature				*Date*	12/19/2003

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Date: December 19, 2003 Express Mail Label No.: EV 117250566 US

Inventor(s): Franklin W. Okumu,
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Attorney's Docket No.: 8024-014-PR

TREATMENT OF DEGENERATIVE CARTILAGE CONDITIONS IN A MAMMAL WITH GLYCOSIDASE INHIBITORS

TECHNICAL FIELD:

The present invention relates to treating, preventing, and lessening the severity of conditions selected from the group consisting of synovitis, subchondral bone edema, and cartilage degradation with administration of glycosidase inhibitors.

BACKGROUND OF THE INVENTION:

Synovitis, subchondral bone edema, progressive cartilage degradation and other similar conditions are separate and distinct conditions of the joints due to, among other things, physical injuries. These conditions may also be associated with osteoarthritis (OA) or rheumatoid arthritis (RA) (Ayral et. al.

Rheumatology, Vol 35, 14-17; McAlindon, Best Pract Res Clin Rheumatol 1999; 13(2):329-44). For example, they may appear as secondary conditions to OA or RA or on their own due to other injuries. Osteoarthritis is the most common joint disease, currently affecting approximately 40 million Americans. This number is expected to increase to 60 million within the next 20 years. Diverse risk factors contribute to the multifactorial etiology of OA. The central pathogenetic mechanism in OA is an aberrant cartilage matrix remodeling process with loss of cartilage cells and matrix, resulting in biomechanical joint failure and inflammation.

Common in these conditions is an erosion of the cartilage. Cartilage erosion results from the over-catabolism of glycosaminoglycans (GAGs) of the proteoglycan (PG)-hyaluronate complex, which comprises the bulk of cartilage tissue. It is known that patients with arthritis, for example, have an abnormal increase of beta-N-acetylhexosaminidase activity in the synovial fluid (O. Kida, J. Japan Orthop. Assoc. 1968, 42(6), 4010; R.W. Stephen, et al., Biochim. Biophys. Acta 1975, 399(1), 101; and J.J. Steinberg, et al., Biochim. Biophys. Acta 1983, 757(1), 47). In rheumatoid arthritis, for example, the dominant glycosidases are the hexosaminidases, such as beta-D-N-acetylglucosaminidase and beta-D-N-acetyl-galactosiminidase. These hexosaminidases, acting either alone or in combination with other glycosidases such as beta-D-glucuronidase, were shown to be directly involved in depleting GAGs from cartilage (Z. Ortutay, et al., Arthritis Rheum. 2003, 48(8), 2163). Thus, Applicants have designed and synthesized a specific hexosaminidase inhibitor that was extremely potent,

having a Ki of 24 nM against hexosaminidase and thereby preventing cytokine-induced loss of GAGs in cultured chondrocytes (J. Liu, et al., Chem. Biol. 2001, 8(7), 701). Further studies revealed that this inhibitor could provide a chondroprotective benefit in an osteoarthritis animal model (*vide infra*).

Unfortunately, the prior art does not provide for an effective means of treating, preventing, and lessening the severity of synovitis, subchondral bone edema, and cartilage degradation. Accordingly, there remains a great need for methods to treat, prevent, and lessen the severity of these conditions, which overcomes the shortcomings of the prior art.

BRIEF SUMMARY OF THE INVENTION:

Applicants have determined that inhibition of glycosidases in the synovial fluid has great utility as a novel chondroprotective approach in treating diseases associated with cartilage degredation. Administration of said inhibitors against these targets are useful therapeutic interventions for treating synovitis, subchondral bone edema, cartilage degradation, and other similar conditions. Therefore, inhibition of the glycosidases in synovial fluid has great utility as a novel approach to chondroprotection.

One embodiment of the present invention relates to using glycosidase inhibition to address the inflammatory and cartilage-degrading conditions of joint diseases. Said joint diseases include, but are not limited to synovitis, subchondral bone edema, cartilage degradation, and other similar conditions. According this embodiment, inhibitors of glycosidases such as hexosaminidases

or glucuroinidases can be utilized as chondroprotective agents that interfere with the breakdown of the cartilage matrix of the joint.

In another preferred embodiment of the present invention, an inhibitor of hexosaminidase, demonstrated unexpected chondroprotective effects in mammals. These chondroprotective properties give rationale to utilizing a glycosidase inhibitor(s) as a therapeutic approach for treating, for example, osteoarthritis (OA), or rheumatoid arthritis (RA).

Another preferred embodiment of the present invention contemplates the utilization of a single glycosidase inhibitor in combination with other glycosidase inhibitor(s) and/or another anti-inflammatory drug(s) or aminosugars for treating arthritis.

A preferred embodiment of the present invention relates to methods of treating, preventing, and lessening the severity of synovitis, subchondral bone edema, and cartilage degradation by administering to a patient a therapeutically effective amount of a glycosidase inhibitor, such as a hexosaminidase inhibitor, or glucuronidase inhibitor or a combination thereof. A therapeutically effective amount of the inhibitors can be administrated to a patient by any means well known in the art, including, but not limited to orally, intravascularly, intra muscularly, topically or intra-articularly. A therapeutically effective amount of such inhibitors may also be administered intra-articularly in a matrix as a controlled release or sustained release formulation.

Another preferred embodiment of the present invention, the invention relates to a method including administering to a patient a composition containing

a therapeutically effective amount of a glycosidase inhibitor (such as a hexosaminidase inhibitor(s) or a glucuronidase inhibitor(s) or a combination thereof), either alone or in combination with an existing anti-inflammatory drug or other therapeutic molecule. Methods of administering formulations of the present invention include, but are not limited to, intravascular, intra-articular, topical, oral, and intra-muscular methods.

In one embodiment of the method, a combination of glycoside inhibitors having a specific activity or a variety of activities against hexosaminidase, glucuronidase or other endo- and exoglycosidases may also be used to achieve a chondroprotective effect in the joint.

BRIEF DESCRIPTION OF THE DRAWINGS:

Figure 1 shows the Femur Lesion Grades 8 weeks after anterior cruciate ligament transection (ACLT) surgery for animals treated with saline or a hexosaminidase inhibitor ((2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine). Error bars represent one standard deviation.

Figure 2 shows the Tibial Lesion Grades eight (8) weeks after ACLT surgery for animals treated with saline or with the specific hexosaminidase inhibitor ((2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine). Error bars represent one standard deviation.

Figure 3 shows the Joint Swelling Grading 8 weeks after ACLT surgery for animals treated with saline or with the specific hexosaminidase inhibitor ((2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine). Error bars represent one standard deviation.

Figure 4 shows the Synovial Fluid Grading 8 weeks after ACLT surgery for animals treated with saline or with the specific hexosaminidase inhibitor ((2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine). Error bars represent one standard deviation.

Figure 5 shows the effect the the specific hexosaminidase inhibitor ((2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine) has on the prevention of IL-1.beta.-induced sGAG loss. Error bars represent one standard deviation.

DETAILED DESCRIPTION OF THE INVENTION:

Applicants have determined that inhibition of glycosidases in the synovial fluid has great utility as a novel chondroprotective approach in treating diseases associated with cartilage degredation. Administration of said inhibitors against these targets are useful therapeutic interventions for treating synovitis, subchondral bone edema, cartilage degradation, and other similar conditions.

Therefore, inhibition of the glycosidases in synovial fluid has great utility as a novel approach to chondroprotection.

For example, accelerated loss of proteoglycans and glycosaminoglycans is a hallmark of osteoarthritic cartilage. Increased catabolism of proteoglycans and glycosaminoglycans compromises both the functional and structural integrity of the cartilage matrix and eventually renders the tissue incapable of resisting the compressive loads applied during joint movement (Inerot, S., et al., Biochem. J., 1978, 169(1), 143). Over time, this process leads to irreversible cartilage degeneration. Loss of articular proteoglycans in established joint disease could be more significant than the collagen loss (Mankin, H.J. et al., 1970 J. Bone Joint Surg. Am., 52(3), 424). In addition to quantitative changes, affected cartilage also undergoes certain qualitative changes. Among these changes are a disproportionately increased ratio of chondroitin 4-sulfate to chondroitin 6-sulfate; a decreased ratio of keratan sulfate to chondroitin sulfate (Mankin, H.J., et al., 1971, J. Clin. Invest. 50(8: 1712); and a decreased sulfation of the terminal residues in chondroitin and dermatan sulfate chains (Plaas, A.H., et al., 1998, J. Biol. Chem. 273(20), 12642).

Degradation of the cartilage matrix is a multifactorial process involving the degradation of glycosaminoglycans by the glycosidases and involving the action of several metalloproteinases, such as collagenases, stromelysins, aggrecanases, and cysteine proteases such as cathepsins. (Winchester, B.G., 1996 Subcell.Biochem. 27, 191; Kresse, H., et al. 1987, Adv. Enzymol. Relat. Areas Mol. Biol., 60, 217).

Glycosidases are produced by chondrocytes and possess an enzymatic activity towards several glycosaminoglycans. Hexosaminidase is a dominant glycosaminoglycan-degrading glycosidase released by the chondrocytes into the extracellular compartment, and it is the dominant glycosidase in synovial fluid of patients with osteoarthritis (Shikhman, A. et al., 2000 Arthritis Rheum. 43, 1307).

Hexosaminidase belongs to the group of lysosomal hydrolases. Hexosaminidase catalyzes the hydrolysis of terminal, non-reducing N-acetyl-.beta.-D-glucosamine and N-acetyl-.beta.-D-galactosamine residues in glycoproteins, G.sub.M2-gangliosides, and glycosaminoglycans, including chondroitin 4-sulfate, chondroitin 6-sulfate, hyaluronic acid, keratan sulfate and dermatan sulfate (Winchester, B.G. 1996 ibid.).

The present invention provides examples to teach how the utilization of a glycosidase inhibitor (such as a hexosaminidase inhibitor) can produce a desired chondroprotective effect in a pathologic condition of cartilage such as synovitis, subchondral bone edema and cartilage degradation. This approach represents a novel means to prevent cartilage matrix glycosaminoglycan degradation and a new strategy for treating osteoarthritis, rheumatoid arthritis, inflammatory joint diseases, traumatic joint diseases and related pathologic conditions.

Abbreviations and Terms:

In accordance with the present invention and as used herein, the following terms and abbreviations are defined with the following meanings, unless explicitly stated otherwise. These explanations are intended to be exemplary only, and

are not intended to limit the terms as they are described or referred to throughout the specification. These explanations include any additional aspects and/or examples of the terms as described and claimed herein.

GAGs = glycosaminoglycans;

HA = hyaluronic acid;

IL-l.beta. = interleukin-l.beta.;

IL-6 = interleukin-6;

OA = osteoarthritis;

RA = rheumatoid arthritis;

sGAG = sulfate-containing form of glycosaminoglycan

The term "therapeutically effective amount" refers to the amount of a biologically active substance necessary to induce a desired pharmacological effect. The amount can vary greatly according to the effectiveness of a particular active substance; the age, weight, and response of the individual; as well as the nature and severity of the individual's symptoms. Accordingly, there is no upper or lower critical limitation with respect to the amount of the active substance. A therapeutically effective amount to be employed in the present invention can readily be determined by those ordinarily skilled in the art.

The term "aminosugar" refers to any synthetic or naturally occurring sugar wherein one or more carbon atoms are substituted with an amino group (-NR.sup.1R.sup.2). Such substitution may occur without regard to orientation or

configuration of any asymmetric carbons present in the sugar. Unless stated otherwise, the term "aminosugar" refers to either anomer (alpha or .beta.) of a cyclic or open chain amino sugar. Aminosugars may be N-substituted with alkyl or acyl group, where one hydrogen atom of a pendant amino group is replaced by an alkyl or acyl moiety (-COR where R = lower alkyl).

The term "arthritis" refers to any particular disease characterized by joint inflammation, although the etiology of the inflammation may differ in various conditions. Relatively common arthritic diseases include rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, psoriatic arthritis, traumatic arthritis and osteoarthritis. Osteoarthritis is also referred to as "degenerative joint diseases."

The terms "articular cartilage" or "cartilage" refer to a substance that covers ends of bones and forms the joint surfaces. Cartilage can withstand compressive forces and creates a low friction surface for a joint to glide on. Articular cartilage comprises chondrocytes and a substrate comprising proteins and glycosaminoglycan polysaccharides.

The term "cartilage degradation" refers to degradation in the tissues comprising cartilage.

The term "chondrocyte" refers to cells found within articular cartilage. Chondrocytes produce collagen, a gelatinous protein, and proteoglycans, which are glycosaminoglycans linked to proteins (also called mucopolysaccharides).

The term "less severe" refers to a particular grade in cartilage degradation of patient. Preferably, less severe grade is in the range of Grade 1 to Grade 3. More preferably, less severe grade is in the range of Grade 1 to Grade 2.

The term glucuronic acid refers to a derivative of D-glucose with a CO.sub.2H group at the C-5 position that is a major component of GAGs.

The term glucuronidase refers to an enzyme which releases a glucuronic acid residue from a substrate such as GAG.

The term "glycosaminoglycan" refers to long heteropolysaccharide molecules containing repeating disaccharide units. The disaccharide units may comprise normal modified amino sugars: D-N-acetylgalactosamine or D-GlcNAc and an uronic acid such as D-glucuronate or L-iduronate. Among other functions, GAGs serve as a lubricating fluid in the joints. Specific GAGs of physiological significance are hyaluronic acid, dermatan sulfate, chondroitin sulfate, heparin, heparan sulfate, and keratan sulfate.

The term "hyaluronic acid" refers to a naturally occurring linear polysaccharide formed by repeating disaccharide units consisting of D-glucuronic acid .beta.(1-3) N-acetyl-D-glucosamine linked by .beta.(1-4) glycosidic linkages.

The term "IL-1.beta." refers to interleukin-1.beta., an immunomodulator that mediates a wide range of immune and inflammatory responses, including the activation of B- and T -cells.

The term "intra-articular" refers to a method of delivering a drug directly to a joint. Traditional routes of drug delivery, such as for example, oral, intravenous or intramuscular administration, depend upon vascular perfusion of the synovium to carry the drug to the joint. This is inefficient because transynovial transfer of small molecules from the synovial capillaries to the joint space generally occurs by passive diffusion, which becomes less efficient with increasing size of the

target molecule. Thus, the access of directing molecules, for example, glucosamine, to the joint space is substantially restricted. Intra-articular injection or perfusion of drugs circumvents such limitations.

The term "sustained release" refers to the time period during which a drug is released for availability, or otherwise becomes available for physiological uptake. Periods of sustained release may be preceded by an induction period, during which little or no drug is released, or may be biphasic, comprising an initial time period during which some drug is released, and a second time period during which additional drug is released.

In contrast, the term "continuous release" is used solely to describe a release profile that appears to be monophasic, having a smooth-curved time profile of release. Those of skill in the art will appreciate that the release profile may actually correspond to an exponential or logarithmic time-release profile.

Examples:

The present invention will be described in more detail by the following examples, which should not be construed as limiting the present invention.

Example 1

Effect of Continuous Infusion of a Hexosaminidase Inhibitor in an Osteoarthritis Animal Model.

The model used in this study is the transection of the anterior cruciate ligament (ACL) in the rabbit knee. ACL transection (ACLT) causes joint instability and subsequent development of degradative and osteoarthritis-like changes.

Female New Zealand White rabbits, 3.0-3.5 kg, were used and were randomly allocated into groups of 8 rabbits. Group A was the saline-treated control group; Group B was treated with 30 mM of the hexosaminidase inhibitor (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine. All the compounds were delivered by a 2ml Alzet osmotic pump (Alzet 2ML4, Alza, USA). The delivery rate from the pump was 2.5 microlitersl/hour. All rabbits are received ACLT surgery on the right knee.

All rabbits were anesthetized by an intramuscular injection of ketamine (35 mg/kg) and acepromazine (2.5 mg/kg). Knees were shaved and disinfected with Antibex (Vetoquinol S.A.) solution. A medial parapatellar incision was made on the skin and a medial arthrotomy was performed. The patella was dislocated laterally and the knee was placed in full flexion. The ACL was visualized and transected with a #15 blade. Complete transection was confirmed by a manual anterior drawer test. The joint was irrigated with sterile saline then closed. Before the closure of knee joint, an Alzet pump prefilled with compounds (either with saline or with the hexosaminidase inhibitor (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine) was implanted subcutaneously in the lower right abdomen of rabbits. The pump was connected by polyethylene tubing (ID: 0.58 mm), which was inserted into the right knee joint

with its tip resting in the synovial space. The joint capsule was closed with a running suture and the tubing connecting the pump was also fixed into the tissue. The skin was closed with interrupted sutures. The Alzet pumps were replaced with fresh units at the end of the fourth week after the operation.

The animals were sacrificed eight (8) weeks after ACLT. The gross morphological changes of both knees, including joint swelling and joint fluid, are evaluated. The occurrence, site and severity of lesions on the surface of the femurs and tibia were determined during observations under a dissecting microscope using the following criteria: Grade 1 (Intact surface), Surface is normal in appearance and does not retain Indian ink; Grade 2 (Minimal fibrillation), Surface retains India ink as elongated specks or light gray patches; Grade 3 (Overt fibrillation), Areas which are velvety in appearance and retain India ink as intense black patches; Grade 4 (Erosion), Loss of cartilage exposing the underlying bone.

The grading of the joint swelling is as following: 0 (normal); 1 (mild), inflammation and/or proliferation of the joint capsule; 2 (moderate), thickening of joint capsule and/or inflammation of the synovium; 3 (severe) abundant inflammation of the synovium, swelling of the menisci or ligaments (anterior or posterior cruciate ligaments). The grading of the joint fluid is as following: 0 (normal); 1 (mild) Fluid is greater than normal, but does not fill the knee joint; 2 (moderate) Fluid fills the knee joint, but does not pour out of the capsule as it is opened; 3 (severe) Fluid expands the knee joint and pours out as the capsule is opened.

Results in Example

Observations on the knee tissues revealed that treatment with the hexosaminidase inhibitor (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine resulted in consistently less chondro-degenerative pathology to the knee joint, compared to saline-treated animals.

Figures 1 and 2 show that treatment with the hexosaminidase inhibitor (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine leads to a statistically significant decrease in the severity of femur and tibia lesion scores, respectively, compared to saline-treated animals.

Figure 3 shows that treatment with (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine produces a trend towards decreased joint-swelling, compared to saline-treated animals.

Figure 4 shows that treatment with (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine results in normal levels of fluid in the knee joint. In contrast, there were increased effusions in the knees of the saline-treated animals. The scoring difference between the (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine group and the saline-treated group was statistically significant.

Example 2

Reduction of sGAG loss By Continuous Infusion of a Hexosaminidase Inhibitor

Female New Zealand White rabbits, 3.0-3.5 kg, were used and were randomly allocated into groups of 8 rabbits. Under aseptic conditions, 2 ml Alzet osmotic pumps (delivery flow rate: 10 microliter/h, (Alzet 2ML1, Alza, USA)) were filled with IL-1.beta. (1000 U/ml; R&D Systems, USA). Separate 2 ml Alzet pumps were filled with 30 mM of the hexosaminidase inhibitor 2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine or with saline. The Alzet pumps were implanted subcutaneously in the lower abdomen of rabbits, and were connected by a polyethylene tubing (ID: 0.025 in) threaded subcutaneously to the left knee joint with their tips resting in the synovial space. The untreated contralateral knees of all animals served as negative controls. IL-1.beta. and (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine were infused intra-articularly for 7 days. Immediately before surgery, and for 3 consecutive days thereafter, Marbocyl, 6 mg/kg/day, is administered (i.m.) to prevent infection. Animals were harvested on day 7.

Sulfated Glycosaminoglycan Assay

Rabbits were sacrificed by i.v. injection of pentobarbital. Both knee joints were removed and the articular cartilages of lateral tibia plateaus were harvested using a scalpel while visualized under a dissection microscopy. The cartilages were weighed and stored at -80.deg.C. For analysis, the samples were thawed

just prior to the assay. sGAGs were extracted from the sample using freshly prepared papain (1 mg papain in 2 ml of 50 mM phosphate buffer, pH 6.8 (PBS), containing 1.0 M NaCl, 5 mM, cysteine-HCl and 1 mM EDTA). 1 mg cartilage was digested with 20 microliter PBS-papain solution while stirred at 60.deg.C for 24 hrs. After digestion, the samples were centrifuged at 10,000 rpm for 10 min, the supernatants then diluted 75 times with PBS-papain buffer then in the following assay.

Blyscan dye (Biocolor, USA) was added to each tube and vortexed. The solution was mixed at 90 rpm using a mechanical shaker for 30 minutes then centrifuged at 20,000 x g. The pellets were reconstituted with 450 microliters of Blyscan dissociation reagent and vortexed for at least 3 min to fully dissolve the pellets. The absorbance of the sample was then observed at 656 nm using a spectrophotometer. Ultra pure water was used as blank. The absorbance of the blank was subtracted from each sample. Results are expressed as micrograms of sGAG recovered per milligram wet weight of cartilage tissue.

The recoverable sGAG content in the cartilage of the left tibial plateau of untreated knees was about 46 micrograms per milligram of wet weight of tissue (Figure 5). Following infusion, the amount of recoverable sGAG in saline-treated knees was about 28% less than in the non- IL-1.beta.-treated control left knee (33 .micro.g/mg of wet weight of left tibial plateau cartilage vs. 46 .micro.g/mg). In the (2R,3R,4R,5R)-N-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine treated knees, the amount of recoverable sGAG was approximately 41 .micro.g/mg, a 24% increase over saline-treated knees.

CLAIMS:

We claim:

1. A method for curing, treating, preventing or lessening the severity of a condition selected from the group consisting of synovitis, subchondral bone edema and cartilage degradation by the administration of a sufficient quantity of at least one glycosidase inhibitor.

2. The method of claim 1, wherein said cure, treatment, prevention or lessening of the severity of said condition is by the administration of a sufficient quantity of at least one glycosidase inhibitor in a cocktail with a second therapeutic molecule selected from the group consisting of anti-inflammatory agents and aminosugars.

3. The method of claim 1, wherein said cure, treatment, prevention or lessening of the severity of said condition is by the administration of a sufficient quantity of a cocktail comprising at least one glycosidase inhibitor in a cocktail with an anti-inflammatory agent and an aminosugar.

4. The method of claim 1, wherein said glycosidase inhibitor is selected from the group consisting of, glycosidase inhibitors, non-iminocyclitol glycosidase inhibitors, hexosaminidase inhibitors and glucuronidase inhibitors.

5. The method of claim 1, wherein said glycosidase inhibitor has a specific activity against a glycosidase selected from the group comprising, glycosidases, hexosaminidases, glucuronidases, endoglycosidases and exoglycosidases.

6. The method of claim 1, wherein said condition results in osteoarthritis.

7. The method of claim 1, wherein said condition results in rheumatoid arthritis.

8. The method of claim 1, wherein said condition results in arthritis.

9. The method of claim 1, wherein said condition results in inflammatory joint disease.

10. The method of claim 1, wherein said condition results in traumatic joint disease.

11. The method of claim 1, wherein said administration is by a route selected from the group consisting of oral, intra-vascularly, intra-articularly, intra-muscularly and topically.

12. The method of claim 1, wherein said glycosidase inhibitor is formulated in a sustained or controlled release formulation.

13. The method of claim 1, wherein said glycosidase inhibitor is delivered to the disease site using a delivery device.

14. The method of claim 13, wherein said delivery device is an Alzet Pump

15. A method for curing, treating, preventing or lessening the severity of an inflammatory condition by the administration of a sufficient quantity of at least one glycosidase inhibitor.

16. The method of claim 15, wherein said administration is by a route selected from the group consisting of oral, intra-vascularly, intra-articularly, intra-muscularly and topically.

17. The method of claim 15, wherein said glycosidase inhibitor is formulated in a sustained or controlled release formulation.

18. The method of claim 15, wherein said glycosidase inhibitor is delivered to the disease site by a delivery device(s).

19. The method of claim 18, wherein said delivery device is an Alzet pump.

ABSTRACT OF THE DISCLOSURE

This invention relates to treating, preventing, and lessening the severity of conditions selected from the group consisting of synovitis, subchondral bone edema, and cartilage degradation with administration of glycosidase inhibitors.

Title: TREATMENT OF DEGENERATIVE CARTLAGE CONDITIONS
IN A MAMMAL WITH GLYCOSIDASE INHIBITORS
Inventor: Franklin W. Okumu, et al.
Attorney Docket 8014-014-PR
Drawing Sheet 1 of 5

Figure 1

Femur Lesion Grading
Hexosaminidase Inhibitor (OPT-66) vs. Saline

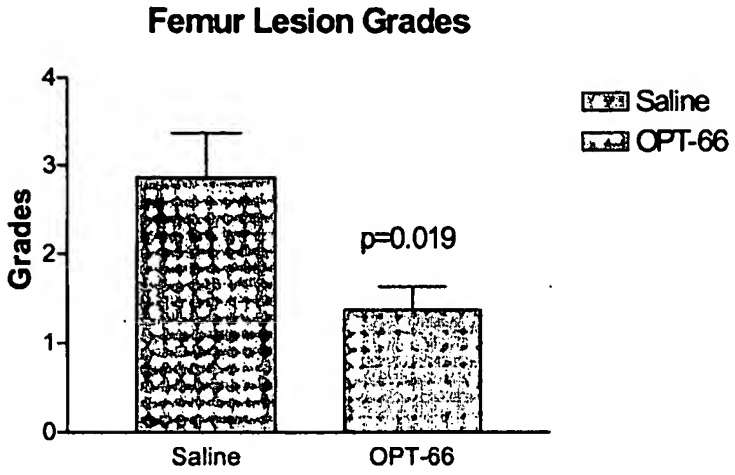


Figure 2
Tibia Lesion Grading:
Hexosaminidase Inhibitor (OPT-66) vs. Saline

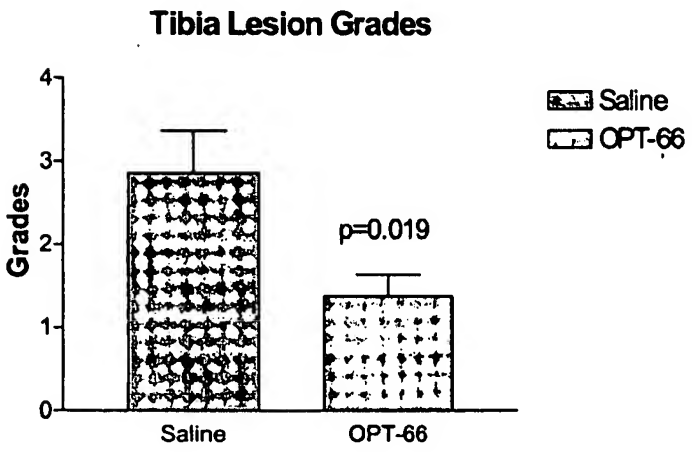
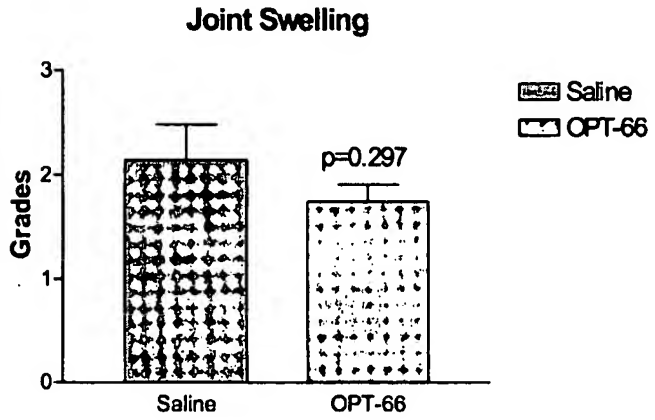


Figure 3
Joint Swelling Grading:
Hexosaminidase Inhibitor (OPT-66) vs. Saline



Title: TREATMENT OF DEGENERATIVE CARTLAGE CONDITIONS IN A MAMMAL WITH GLYCOSIDASE INHIBITORS

Inventor: Franklin W. Okumu, et al.
Attorney Docket 8014-014-PR
Drawing Sheet 4 of 5

Figure 4
Synovial Fluid Grading:
Hexosaminidase Inhibitor (OPT-66) vs. Saline

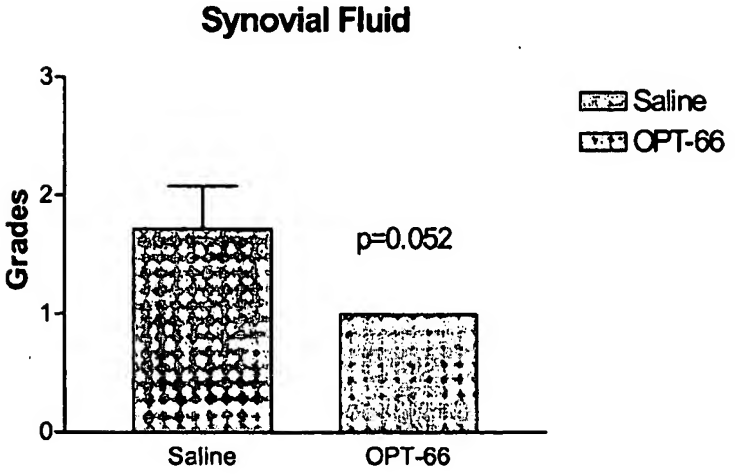


Figure 5

Effect of Hexosaminidase Inhibitor (OPT-66)
On IL-1β-Induced sGAG Loss